Serial No. : 09/981,648

IN THE CLAIMS

24. (previously added) A method for making an infectious adenovirus which comprises contacting a cell with or introducing into a cell:

a. a first nucleic acid sequence being a plasmid comprising a circularized adenovirus DNA molecule having a deletion of an adenoviral packaging signal, and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus; and

b. a second nucleic acid sequence which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, and encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable of encoding an infectious, packageable adenovirus;

provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination may occur between said first and said second nucleic acid sequences, whereby said first and said second nucleic acids recombine to form said infectious adenovirus.

25. (previously added) The method according to claim 24 wherein said adenovirus DNA additionally comprises at least one of (i) a deletion of, or (ii) a modification in, an adenoviral gene selected from the group consisting of adenoviral E1 sequences 3' of said packaging signal, adenoviral fibre gene sequences, adenoviral E3 gene sequences, and adenoviral E4 gene sequences.

26. (previously added) A method for making an infectious adenovirus which comprises contacting a cell with or introducing into a cell:

a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious,

packageable adenovirus; and

b. a second nucleic acid sequence, which is a plasmid formed by combination of (i) at least one of the shuttle plasmids selected from the group consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118, and (ii) a polycloning site or a foreign DNA or an expression cassette, and which second nucleic acid sequence, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus;

provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination may occur between said first and said second nucleic acid sequences, whereby said first and said second nucleic acids recombine to form said infectious adenovirus.

27. (previously added) A recombinant adenovirus vector system comprising:

a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination with homologous sequences in a second nucleic acid sequence may occur; and

b. the second nucleic acid sequence, which is a plasmid formed by combination of (i) at least one of the shuttle plasmids selected from the group consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118, and (ii) a polycloning site or a foreign DNA or an expression cassette, and which second nucleic acid sequence, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus; said second nucleic acid sequence comprising a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences to permit homologous recombination with said first nucleic acid sequence;

whereby said first and said second nucleic acids homologously recombine in a cell to form said infectious adenovirus.

28. (previously added) A recombinant adenovirus vector system comprising:

a. a first nucleic acid sequence encoding adenovirus sequences, and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination with homologous sequences in a second nucleic acid sequence may occur; and

b. the second nucleic acid sequence which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus; said second nucleic acid sequence comprising a head-to-head ITR junction, an adenoviral packaging signal, and sufficient overlapping adenoviral nucleic acid sequences to permit homologous recombination with said first nucleic acid sequence;

whereby said first and said second nucleic acids homologously recombine in a cell to form said infectious, packageable adenovirus, and wherein said cell expresses adenoviral E1.

29. (amended herein) A kit for construction of recombinant adenovirus vectors comprising:

(A) a first nucleic acid sequence encoding adenovirus sequences and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, and said first nucleic acid sequence comprising a head-to-[tail]<u>head</u> ITR junction and sufficient adenoviral sequences to permit homologous recombination with similar sequences in a second nucleic acid sequence;

(B) the second nucleic acid sequence which is a plasmid formed by combination of (i)

at least one of the shuttle plasmids selected from the group consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118, and (ii) a polycloning site or a foreign DNA or an expression cassette, and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, and said second nucleic acid sequence comprising a head-to-head ITR junction and sufficient adenoviral sequences to permit homologous recombination with similar sequences in said first nucleic acid; and

(C) a cell wherein, when said component (A) and said component (B) are cotransfected and recombined through homologous recombination, an infectious recombinant adenovirus vector is produced.

30. (previously added) A recombinant adenovirus vector system comprising:

a. a first nucleic acid sequence comprising a deletion in the adenoviral fibre gene, and encoding other adenovirus sequences, and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination with homologous sequences in a second nucleic acid sequence may occur; and

b. the second nucleic acid sequence which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus; said second nucleic acid sequence comprising a head-to-head ITR junction, an adenoviral packaging signal, and sufficient overlapping adenoviral nucleic acid sequences to permit homologous recombination with said first nucleic acid sequence;

whereby said first and said second nucleic acids homologously recombine in a cell to form said infectious, packageable adenovirus.

Serial No. : 09/981,648

31. (previously added) A recombinant adenovirus vector system comprising:

a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous recombination with homologous sequences in a second nucleic acid sequence may occur; and

b. the second nucleic acid sequence which, by itself, in the absence of intermolecular recombination, is incapable of generating an infectious, packageable adenovirus; said second nucleic acid sequence comprising a head-to-head ITR junction, an adenoviral packaging signal, an adenoviral gene mutation, and sufficient overlapping adenoviral nucleic acid sequences to permit homologous recombination with said first nucleic acid sequence;

whereby said first and said second nucleic acids homologously recombine in a cell to form said infectious, packageable adenovirus, and wherein said adenoviral gene mutation is rescued into said infectious, packageable adenovirus.

32. (previously added) The recombinant adenovirus vector system according to claim 31 wherein said adenoviral gene mutation rescued into said adenoviral vector recombinant is comprised of at least one of a mutation in the adenoviral fibre gene, a mutation in the adenoviral E4 gene, and a mutation in the adenoviral E3 gene.